VIRUS AND CELL INTERACTION WITH ION EXCHANGERS*

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(Received for publication, February 2, 1953)

In earlier work, evidence was presented showing that the attachment of bacterial viruses to their specific host cells involves establishment of electrostatic bonds between sites on the surfaces of the two bodies (1–4). It was demonstrated that union of virus and host cannot occur in distilled water but requires the presence of cations whose charge and optimal concentration range are specific for different viruses. The attachment of T1 and T4 viruses to a cationic exchanger was shown to exhibit specific inorganic and organic cofactor requirements similar to those involved in host-cell invasion. Since only the cations were shown to be important in promoting cell attachment and because other lines of evidence demonstrate that at least some of the T-phages and *Escherichia coli* cells are negatively charged at pH 7 (5, 6), it was postulated that the function of these positive ions is to neutralize repulsive electrostatic forces which otherwise prevent attachment. Further experiments with ion exchange resins are here reported which support this picture, and indicate that similar principles are involved in the case of influenza virus. In addition, it is shown that T2 bacteriophage is split into its P- and S-containing components as a result of its attachment to the negatively charged surface of a cationic exchanger.

Methods and Materials

(*a*) *Bacteriophage Experiments.*—Details of procedures for preparation and titration of the T-system bacteriophages and their host cells have been presented earlier (1). P^{32}- and S^{35}-labelled T2 bacteriophages were prepared by various methods, the method of Hershey (7) giving the best results. The radioactive phage preparations assayed approximately 10^{11} plaque-forming particles per cm.^{3} after purification by 3 cycles of alternating slow and high speed centrifugation and had specific activities of $3 \times 10^{-11}\mu$c./phage and $2 \times 10^{-11}\mu$c./phage for the P^{32} and S^{35} preparations, respectively. All radioactivity assays were performed in multiplicate, with an end window Geiger tube (1.2 mg./cm.^{2}). The over-all precision for each individual assay encompassed an uncertainty of not more than 8 per cent. Between 75 and 90 per cent of the radioactivity of such preparations attached to host cells, *E. coli* B, while only 0 to 15 per cent could be bound to the specifically resistant cell mutant, *E. coli* B/2.

(*b*) *Influenza Virus.*—Influenza PR8 stocks were prepared by growth on allantoic membranes of chick embryos. The allantoic fluid of infected eggs was collected and frozen im-

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

‡ Contribution No. 14.

mediately. Samples were thawed before each experiment and diluted to the concentrations desired in appropriate media. The virus preparations were originally titred for their ability to agglutinate red cells and to multiply in eggs. In most of the experiments here reported, only hemagglutination was employed, using a 0.125 per cent dilution of a packed, chick red cell suspension which was read by means of the pattern procedure (8). Serial twofold dilutions were employed, and the end-point of the titration was taken as the last tube in such a series in which hemagglutination definitely occurs. The reciprocal of this dilution was designated as the hemagglutinating titre of the suspension. Sufficiently large amounts of virus were employed in all experiments so that 8 to 10 of such serial dilution tubes were used in each initial assay. In some experiments hemagglutination was also measured spectrophotometrically by following the optical density of a red cell suspension in a Beckman model B spectrophotometer, using a wave length of 490 mμ. At time designated as 0, the red cell suspension was thoroughly mixed and placed in the spectrophotometer. 6.0 cc. of suspension was employed in a tube 13 × 100 mm. The top of the light slit which was 1.8 cm. high met the tube at a point 2.8 cm. below the surface of the liquid. All assays were performed in multiplicate with excellent checks. The red cells employed were obtained weekly from a slaughter-house, and stored at 0°C. as a 10 per cent dilution of a packed red cell suspension in physiological saline buffered at pH 7.0. All the red cell attachment experiments were conducted at 3°C. to prevent interference by spontaneous elution of influenza virus from the red cells (9).

The ion exchange resins were used in the form of spheres of about 0.5 mm. in diameter. The cationic resin (nalcite RCH)[1] has a sulfonic acid radical as its functional group. Before use it was regenerated by 5 to 10 per cent NaCl, 1 M $MgCl_2$, or 1 M $CaCl_2$, and then washed with double distilled water until washings no longer produced a precipitate when treated with acidified $AgNO_3$. The anionic resin, dowex I,[2] has a strongly basic amino group and it was regenerated with 7.0 per cent Na_2HPO_4. In some experiments the fluid was poured through a column containing the resin, in the conventional procedure. In others, 10 to 40 cc. quantities of virus or cell suspension were added to 5 to 50 gm. of resin (dry weight) in a stoppered bottle which was then agitated, either manually or by a shaking machine. When necessary, appropriate corrections (never more than 15 per cent) were applied to the titres to allow for volume changes due to hold-up fluid entrained with the resin.

RESULTS

1. Demonstration of Negative Charges on the Bacteriophage

T2 bacteriophage which requires a concentration of Na^+ ion of 0.10 M or greater for maximal attachment to its host cell was used in most of these experiments. A $1:10^6$ dilution of the virus stock was prepared in doubly distilled water buffered at pH 7 with 5×10^{-4} M phosphate. This medium permits no measurable attachment of the phage to its host cell, *E. coli* B. 40 cc. of this suspension was added to the cationic exchanger (nalcite R^- Na^+) and to the anionic resin (dowex I). The data of Table I indicate that virus activity disappeared completely from the supernatant liquid bathing the anionic exchanger, but persisted almost unchanged in the presence of the negatively charged resin.

To test whether the addition of NaCl can modify the repulsive potential

[1] National Aluminate Corporation, Chicago.

[2] Dow Chemical Company, Midland, Michigan.

arising from the surface charge of the virus, as was postulated in explaining cell attachment, the tests of Table I were repeated but with 0.15 M NaCl as the suspending medium. Table II presents a typical result of such an experiment, demonstrating that the NaCl in this concentration confers on the virus the ability to attach to the negatively charged surface of the cationic exchanger. Concentrations of NaCl less than 0.1 M resulted in a much slower rate of removal of the virus activity, and in the neighborhood of 0.01 M or less of NaCl the suspension behaved as in distilled water. This behavior closely parallels the attachment of T2 phage to its host cell (1).

TABLE I

T2 Bacteriophage Suspended in Distilled Water at pH 7 Was Shaken for 70 Minutes at 0°C. in the Presence of (a) a Cationic and (b) an Anionic Exchanger

	T2 titre (phage/cm.[3])
Before addition to resin	5500
(*a*) After shaking with cationic exchanger	4000
(*b*) " " " anionic "	0

TABLE II

T2 Bacteriophage Suspended in 0.15 M NaCl, Was Shaken for 30 Minutes with a Cationic and an Anionic Exchanger

	Phage activity in supernatant
	per cent
(*a*) Cationic exchanger	10
(*b*) Anionic "	0

The behavior of the sulfonic acid resins described here is essentially the same as that of inorganic cationic exchangers, like nalcolite zeolite[1] or glass, whose behavior with several viruses was described earlier (1). Similarly, several other anionic exchange resins (amberlite IR4, XE-67)[3] behaved like dowex I in these experiments. Evidently the main features of the behavior described are determined by the charge of the functional group of the resin, rather than its specific chemical nature.

2. Demonstration of Negative Charges on Bacterial Host Cells

Similar experiments carried out with a suspension of *E. coli* B, the specific host cell for the T-system bacteriophages, confirmed the existence of negatively charged binding groups on the cell surface at pH 7. In dilute buffer, the cells become rapidly attached to a positively charged resin, but not to a negatively charged one. However, an important difference from the behavior of the virus

[3] Rohm and Haas, Philadelphia.

lies in the fact that the host cells do not become attached appreciably to a negatively charged resin even in the presence of 0.2 M NaCl (Table III).

3. Difference in Charge Relationships between T1 and T2 Bacteriophages

T1 bacteriophage also attaches to anionic exchangers in distilled water, and will not attach to a negative cationic exchanger except in the presence of salts. As has been described earlier (1), the NaCl requirement for maximal attachment of the T1 virus to a cationic resin is only 0.01 M, a concentration one-tenth that required for T2. A similar parallelism exists in the attachment of these two phages to their common host cell, *E. coli* B (1). It may be concluded that T1 bacteriophage requires a smaller cationic concentration than does T2 to neutralize its excess negative charge sufficiently to permit attachment to a negatively charged surface.

TABLE III

E. coli B Cells Were Shaken with the Two Types of Ion Exchangers in the Media Indicated

Ion exchanger	Medium	Cell titre remaining in supernatant after shaking for	
		3 min.	8 min.
		per cent	*per cent*
Cationic	H_2O	66	81
"	0.15 M NaCl	81	81
Anionic	H_2O	0	0

T2 bacteriophage can utilize 0.1 M $MgCl_2$ or $CaCl_2$ as well as the Na salt for attachment to the cationic resin. For such experiments the resin was regenerated in a 1 M solution of the salt used in the attachment medium in order that the composition of the latter remain constant throughout the test. The results obtained were exactly like those in Tables I and II (lines *a*).

4. Inactivation of T2 Bacteriophage on Cationic Exchangers

Different bacteriophages were found to vary considerably in their ability to be eluted in the viable state, after attachment to a cationic exchanger. It was shown earlier that T1 virus can be almost completely recovered after attachment to a zeolite like glass. Similar results were usually obtained with the sulfonic acid cation exchanger. It never proved possible, however, to elute significant quantities of active T2 phage after attachment either to organic or inorganic cationic exchangers (Table IV).

Experiments with radioactively labelled T2 phage were carried out to determine the fate of the T2 virus after attachment to the resin. The following results reveal that almost immediately after adsorption to the resin T2 phage

is split into at least two fractions, one of which carries the sulfur label and the other most, if not all, of the phosphorus.

(*a*) Although the plaque-forming activity of the T2 phage is never restored, the radioactivity can be recovered almost completely when the phage has been labelled either with P^{32} or S^{35}. Despite reelution of the radioactivity, the characteristic blue Tyndall scattering of the concentrated phage suspensions disappears irreversibly on contact with the resin surface. This observation suggests that the phage has been fragmented into particles too small to scatter blue light.

(*b*) Spontaneous elution of both P^{32} and S^{35} radioactivity was observed in the original resin bottle containing the phage suspended in the attachment medium. Since it was considered possible that the phage particles, having become attached to the resin, might be fragmented by the shearing forces

TABLE IV

Attempts to Elute T1 and T2 Bacteriophage from Cationic Resin, Nalcite R^-Na^+, after Attachment in Medium of Proper Salt Concentration

	Fraction of original T_2 bacteriophage removed after shaking with resin	Fraction of lost bacteriophage activity recovered on washing resin with eluting liquids indicated:
	per cent	*per cent*
(*a*) T2 bacteriophage.....	95	Distilled water, **0.6**
	100	5 per cent NaCl, **0**
(*b*) T1 bacteriophage.....	87	Distilled water, **65**

arising from the vigorous shaking of the mixture on the shaking machine, an experiment was run in which the mixture was agitated so gently as to preclude the generation of mechanical forces capable of rupturing the phage:—

P^{32}- and S^{35}-labelled T2 phages were each added to Ca-regenerated nalcite resin, in a medium containing 0.10 M $CaCl_2$ buffered to pH 7 with 10^{-4} M phosphate. Each resin bottle was agitated very gently by turning it upside down 100 times at a rate of about 1 turn per second. Samples of the supernatant were then plated for phage activity and radioactive content. The agitation and sampling procedures were repeated several times. Typical results of such an experiment are presented in Fig. 1. They reveal that initially both types of radioactivity are taken up from the suspension as the phage activity disappears. But shortly thereafter, the P^{32} is spontaneously and almost completely restored to the liquid phase. The S^{35} activity also elutes spontaneously, but less rapidly and completely than the P^{32}. In some experiments, as much as 93 per cent of the P^{32} was restored to the solution by continuation of this mild agitation.

If the resin is shaken more vigorously, or if it is rinsed with distilled water or even with a new batch of the original suspension medium, most of the remaining S^{35} activity can also be recovered. Usually about 50 per cent of the

S^{35} fraction elutes spontaneously after only mild agitation, and an additional 20 to 30 per cent more is recoverable by further washing of the resin.

Essentially similar results in this type of experiment were obtained for cationic exchange resins regenerated in NaCl, $MgCl_2$, or $CaCl_2$.

(*c*) The two radioactive fractions recovered in the manner described were tested for their ability to attach to host cells, since it has been shown that the S-containing protein portion of the phage retains its ability to attach to host

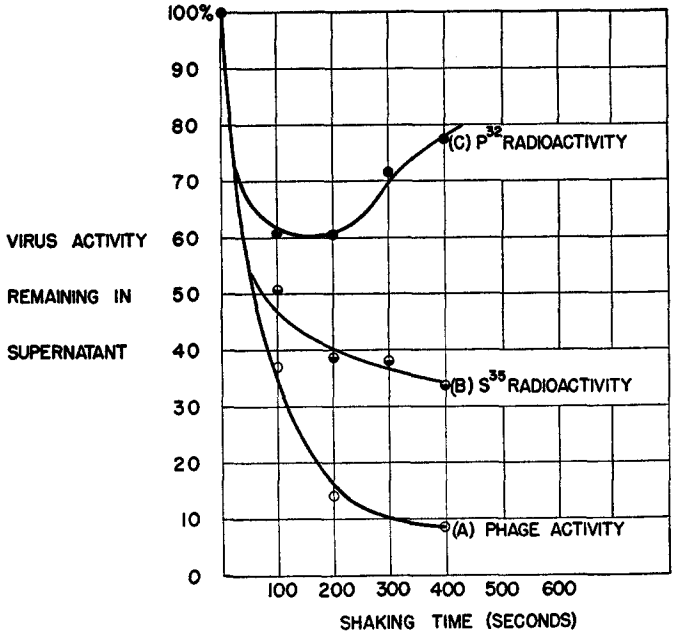

FIG. 1. *A*, the change in plaque-forming titre; *B*, S^{35} radioactivity; and *C*, P^{32} radioactivity of an isotopically labelled phage suspension shaken with a negatively charged resin in 0.10 M salt solution.

cells, but the DNA in which the phage phosphorus is bound cannot so attach once it has split off from the protein (7). The experiments which are summarized in Table V show that the S^{35} fraction obtained from the cationic exchanger has retained its ability to attach to the host, whereas the P^{32} radioactivity shows practically no uptake by the cells.

(*d*) Tests with desoxyribonuclease were conducted to determine whether the P^{32} component of T2 phage liberated by the cation exchanger is indeed free DNA. Samples of a P^{32}-labelled T2 suspension taken before and after exposure to a cationic exchanger were incubated for 30 minutes at 37°C. in the presence of 1 mg./cc. of crystalline desoxyribonuclease[4] suspended in veronal buffer at pH 8 (7). The data of Table V *a* indicate that contact with the ex-

[4] Worthington Biochemical Sales Co., Freehold, New Jersey.

changer converts more than 75 per cent of the P^{32} to a form which is sensitive to the action of the enzyme.

TABLE V

Attachment to Host Cells of Radioactivity Recovered from Cationic Resins

T2 bacteriophage preparations labelled with P^{32} and S^{35}, respectively, were shaken with a cationic exchanger until 90 to 99 per cent of the plaque activity had disappeared. The radioactivity in the original supernatant plus that recovered by treatment of the resin with distilled water was tested for attachment to young cells of *E. coli* B at 37°C. in 0.10 M NaCl. The results of 3 typical experiments are presented.

	Total amount of radioactivity recovered in original supernatant and subsequent washing in distilled water	Fraction of recovered activity adherent to host cells
	per cent	*per cent*
P^{32}	81	3.0
	89	6.5
	115	4.9
S^{35}	67	60.0
	65	81.0
	64	40.0

TABLE V *a*

Test of P^{32} Fraction with Desoxyribonuclease

P^{32}-labelled T2 was shaken with nalcite RC^{-} Mg^{++} for approximately 10 minutes. The supernatant contained less than 0.5 per cent of its original phage activity, but approximately 80 per cent of the radioactive label. Samples of the suspension before and after contact with the resin were incubated at 37°C. with the specific enzyme, chilled to 0°C., treated with 5 per cent trichloroacetic acid, and centrifuged 5 minutes at 2500 R.P.M. in an International centrifuge model PR-1.

Sample of T2 labelled with P^{32}	Counts/sec./cc.	Activity remaining in supernatant after digestion with desoxyribonuclease and precipitation with 5 per cent TCA	
		Counts/sec./cc.	Fraction of initial activity
			per cent
(*a*) Before shaking with resin	222	7.5	3
(*b*) After " " "	178	135.0	76

Control tests in which the enzyme was omitted resulted in removal of 99 per cent of the radioactivity from the supernatant remaining after TCA precipitation and centrifugation, in both cases.

It can be concluded that T2 bacteriophage is split into its free DNA and protein components as a result of its attachment to a cationic exchanger in a medium of appropriate ionic composition.

5. Attachment of Influenza Virus to Ion Exchange Resins

Similar experiments were carried out with influenza virus. An allantoic fluid suspension was diluted in iso-osmotic sucrose, and aliquots added to the

nalcite cationic resin and to the dowex I anion exchanger at 4°C. The flasks were shaken for 30 seconds every 15 minutes and the supernatant fluid sampled periodically and tested for virus content. In accord with the results of the bacteriophage experiments, it was found that no measurable virus removal occurred in the cationic flask after 4 hours, whereas practically all the virus activity disappeared from the supernatant in the flask with the anionic exchange resin within 90 minutes. Similarly, when the virus was suspended in

TABLE VI

Attachment of Influenza Virus to Ion Exchange Resins at 4°C.

Ion exchanger	Suspending medium	Hemagglutinating activity		Disappearance of hemagglutinating activity
		Initial	Final	
				per cent
(*a*) Cationic-nalcite	10 per cent sucrose	128	128.0	0
(*b*) Anionic-dowex I	10 " " "	128	4.0	97
(*c*) Cationic-nalcite	0.85 " " NaCl	128	0.0	100
(*d*) Anionic-dowex I	0.85 " " "	128	4.0	97

TABLE VII

Elution of Influenza Virus Attached to Cationic Exchangers

Influenza virus PR8, suspended in 0.85 per cent NaCl in an amount equivalent to 128 hemagglutinating units/cm.[3] was added to nalcite $RC^- Na^+$, and shaken at 3°C. After the shaking period, the supernatant was assayed and found to contain no hemagglutinating activity. The resin was then washed twice in 0.85 per cent NaCl. The rinse liquid contained no hemagglutinating activity. The elution of virus from such resin was then tested using distilled water and 10 per cent NaCl as eluents, in parallel washing experiments.

Elution medium	No. of hemagglutinating units/cc. eluted	Fraction of original virus activity eluted
		per cent
10 per cent NaCl (3 successive washings)	76	59
Distilled water (1 washing)	32	25

0.85 per cent NaCl, a medium which permits its attachment both to red blood cells and chick fibroblasts, the cationic resin was highly effective in removing the virus from suspension. Table VI summarizes a typical experimental series, demonstrating this similarity in behavior with the T1 and T2 bacteriophages.

6. Elution of Influenza Virus from Cationic Exchangers

Tests on the elution of influenza virus from cationic exchangers revealed that a large part of the attached virus can be recovered in a form which is active in hemagglutinating chicken red cells (Table VII). Muller and Rose (10) have similarly demonstrated that a large portion of the influenza virus

so eluted retains its egg infectivity. It may then be concluded that the attachment of influenza virus to cationic exchangers resembles the reversible binding of T1 rather than T2.

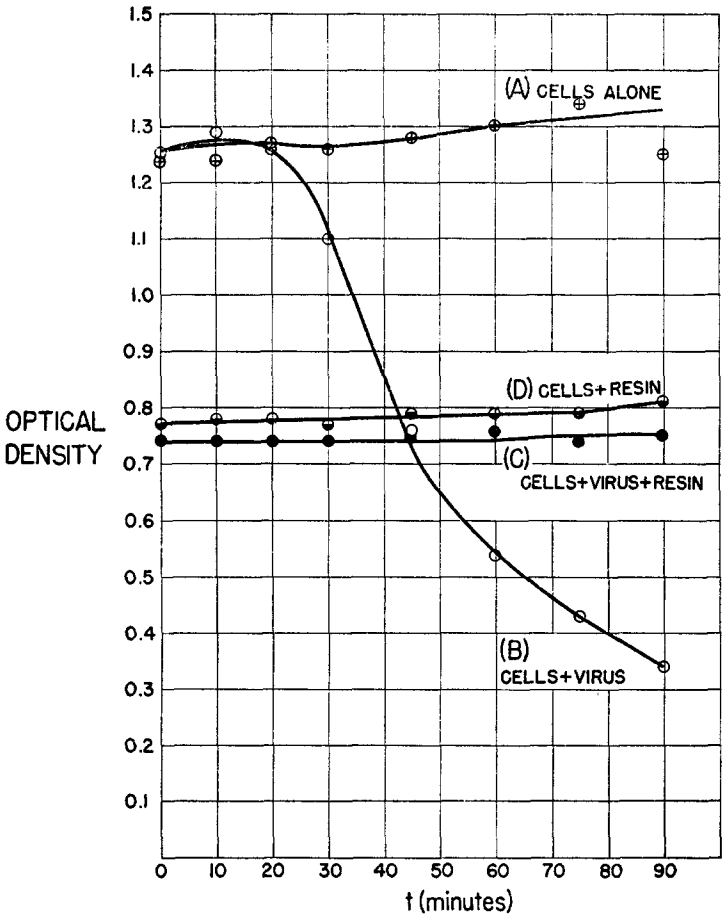

FIG. 2. The time course of hemagglutination of chicken red cells, as followed spectrophotometrically: *A*, cells never exposed to influenza virus; *B*, cells saturated with influenza virus; *C*, cells saturated with virus and then shaken with a cation exchanger in 0.85 per cent NaCl; *D*, control cells never exposed to influenza virus, shaken with cation exchanger in 0.85 per cent NaCl.

7. Removal of Influenza Virus from Red Cells by a Cation Exchanger

Experiments were also performed to determine whether the affinity of viruses for ionic sites on these synthetic compounds is great enough to remove a virus from a cell to which it has become reversibly attached. A direct experimental test of this point would be difficult to interpret because approximately 20 to

50 per cent of the red cells themselves attach to a cationic resin; thus in attempting to elute any virus which had transferred from cells to resin, virus still attached to such cells would also come off. Hence, an experiment was devised to determine whether the surface of a cell which is saturated with influenza virus can be restored to its normal state as a result of exposure to a cation exchanger. Fowl erythrocytes were exposed at 4°C. to a large excess of influenza virus so that all the available active surface on the red cell was saturated, as evidenced by the inability of such cells to adsorb additional virus. These cells agglutinate very strongly. The time course of agglutination can be followed spectrophotometrically, and can be readily reproduced in the same sample, simply by stirring the suspension. These cells were then centrifuged and resuspended in 0.85 per cent NaCl. Aliquots were added to a cationic exchange resin at 4°C., and given 8 shaking periods of 30 seconds each at 15

TABLE VIII

Test of the Ability of Virus-Saturated Red Cells to Attach New Influenza Virus after Exposure to a Cationic Exchanger

Previous treatments	Virus added (hemagglutinating units)	Virus remaining unattached to cells	Fraction of virus attached to cells
			per cent
Cells, fresh	192	12	94
" saturated with virus	192	192	0
" " " " , shaken with resin	192	48	75

minute intervals. As a control, another aliquot of these cells was shaken in a similar fashion in the absence of the resin. Samples of these cells were then collected, and their agglutination time curves measured spectrophotometrically and compared with each other and with the curve of fresh cells. The results are presented in Fig. 2. They reveal that virus-saturated red cells completely lose their agglutinating ability when shaken with a cation exchanger, in 0.85 per cent NaCl at 4°C., whereas shaking in the absence of the resin has no effect on their settling.

To determine whether this loss of agglutination is indeed due to removal of the cell-attached virus, the cells were tested to see whether the resin treatment had restored their ability to attach new virus. In contrast to their behavior before the resin treatment, these cells were now found to take up a fresh supply of influenza virus almost as rapidly as erythrocytes which had never before been exposed to a virus suspension (Table VIII).

It may be concluded that the cationic exchanger in the appropriate ionic medium has a sufficiently great affinity for influenza virus to remove it from red cells to which it has adsorbed.

DISCUSSION

Demonstration of the necessity for salts in promoting attachment of bacteriophages to cationic, but not to anionic exchangers, bears out the previous hypothesis concerning the role of ions in virus cell attachment. The parallelism involved in the fact that T2 bacteriophage requires ten times as high a salt concentration as does T1 for attachment to the resin, just as it does in host cell attachment, constitutes further evidence in addition to that of previous experiments (1) that the cationic resin can serve as a model for the host cell surface with respect to initial attachment of the virus.

It follows that two conditions must be fulfilled in order for virus-host attachment to occur: (*a*) The electrostatic repulsive potential between the two surfaces must be reduced sufficiently to allow the bodies to approach within bonding distance. (*b*) The two surfaces must have complementary structures between which bond formation can occur. Presumably the first condition is effected through the agency of the cations in the medium. Whether this action requires actual binding of cations to the phage surface, or whether simple screening through double layer formation is involved is not yet known. Studies from this laboratory on T1 bacteriophage (11) indicate that divalent cations can enter into direct chemical binding with this phage while monovalent ions act only through double layer formation. The second factor in attachment will be determined by the number and distribution pattern of binding groups on the two surfaces. The nature of the pH influence on cell attachment of T2 bacteriophage suggests that cellular carboxyl groups and virus amino groups take part in the binding (3). Since some groups of bacteriophages with different host-range specificities for attachment have considerably different cationic attachment requirements (*e.g.* T1, T2, and T3), while others have uniform salt requirements throughout the group (T2, T2h, T4), it seems likely that both of the factors governing attachment are involved in virus-host specificity.

Of considerable interest is the splitting of the T2 phage into its protein and DNA components almost immediately after its attachment to the resin. The experiments of Hershey and Chase (7) established that such a separation occurs soon after the virus attaches to its host cell, but threw no light on the mechanism of the process. The fact that a similar splitting is obtained *in vitro* by the action of a violent osmotic shock is not helpful, since it is most unlikely that such extreme conditions could be duplicated at the cell surface.

The present experiments indicate that the P^{32}-containing part of the phage begins to separate from the rest within a matter of seconds after its attachment to the resin. Since there is no doubt that resin attachment consists in electrostatic bond formation between positive groups on the virus and the negative sulfonate radicals, the splitting must occur as a direct consequence of this ionic interaction. Evidently some positively charged component of the virus attaches to the negative sulfonate groups on the resin, and in so doing permits

escape of the phosphorus- and sulfur-containing moieties. Since the P^{32} is released first and in highest concentration, it must be bound to this positive component less tightly than the sulfur. If one accepts Hershey's model of an injection of the DNA through the tail, it is possible that the initial attachment triggers the opening of the tail, which permits the phosphorus fraction to escape. If this picture is correct, one can readily understand how the same splitting will occur at the cell surface as at the resin, since the same train of events will be initiated when the virus becomes attached to the negatively charged groups in either case.

Since both the S^{35}- and P^{32}-containing moieties spontaneously elute from the resin, it may be supposed that some positively charged fragment of the virus still remains attached to the resin. The nature of such a positively charged component is at present unknown. However, since all of the P^{32} and at least a large fraction of the S^{35} separate spontaneously from the resin, regardless of whether the resin has been regenerated in Na^{+}, Mg^{++}, or Ca^{++}, it may be concluded that the component in question is not one of these inorganic ions, at least in simple form. (It could be part of a complexly bound aggregate.) In a forthcoming paper, evidence will be presented that a positively charged substance can initiate an enzymatic step at the cell surface which may make possible penetration of the DNA.

The fact that the S^{35} fraction released from the resin attaches to host cells at a somewhat reduced rate as compared with normal cells is reminiscent of the behavior of the S^{35}-containing fraction which Hershey recovered from infected cells by agitation in a Waring blendor. The simplest explanation for this reduced cell affinity is that the S-fraction has retained a part, but not all, of the positively charged groups by which attachment either to the cell or to the cationic exchanger is made. The properties of both the fractions liberated from the resin are receiving further study.

The greater resistance of T1 bacteriophage to splitting on a cationic exchanger is surprising in view of its greater sensitivity to inactivation by heat, low pH, and solutions of low ionic strength. Experiments are under way to determine whether conditions cannot be found to effect its splitting also, as a result of attachment to a negative surface. The greater ease of the T2 splitting may account for the fact that it is much more difficult to adjust conditions whereby T2 attaches reversibly to its host cell than is the case with T1.

In contrast to the action of T1 and T2 bacteriophages, the failure of bacterial host cells to attach to cationic exchangers even in the presence of fairly high concentrations of salt is of interest. It suggests that while both virus and cell are negatively charged in distilled water at pH 7, the surface of the virus is more readily brought close to neutrality by the cationic components of the medium. Studies of the chemistry and distribution of these charged surface

groups should go far to elucidate the detailed nature of attachment and the physicochemical basis for its specificity.

The similarities in the action of influenza virus and T1 and T2 bacteriophages toward anionic and cationic exchange resins strongly suggest that the principles governing the attachment of bacteriophages to their host cells also apply to mammalian virus systems. Other similarities in these two systems with respect to salt requirements, etc., have been emphasized most recently by Burnet (12). The influenza virus seems to resemble T1 more than T2 bacteriophage in its greater resistance to splitting by the cationic exchanger.

The experiments described here elucidate the conditions under which viruses may become attached to anionic and cationic exchangers. For viruses which are not split by resins, this procedure is useful for concentration and purification, as has been shown for poliomyelitis virus (13), T1 bacteriophage (1), and influenza virus (10). These principles may also have application in the removal of virus contaminants from fluids like blood and serum, where it is immaterial whether the virus is inactivated by the resin.

SUMMARY

At pH 7 the bacteriophages T1 and T2 attach to anionic exchangers when suspended either in distilled water or in salt solutions. They attach to cationic exchangers only in the presence of salt. T2 requires approximately ten times higher a salt concentration than does T1 virus, a relationship which is duplicated in the attachment of these phages to their common host cell, *E. coli* B.

The host cell, *E. coli* B, attaches to anionic exchangers when suspended either in salt or distilled water, but does not attach to cationic exchangers even in 0.2 M NaCl.

These observations support the hypothesis previously advanced that the role of cations in effecting union of viruses to their host cells is to neutralize the repulsive electrostatic potential due to the excess of negative charges on the surfaces of both bodies.

Influenza virus similarly requires salt for attachment to cationic exchangers, and unites to anionic exchangers even in the absence of salt. It is therefore proposed that the same general principles which govern the behavior of the bacteriophage system, may also apply to host cell attachment of at least some mammalian viruses.

T1 bacteriophage and influenza virus are readily eluted from cationic exchangers. T2 bacteriophage cannot be recovered in active form.

Experiments with P^{32}- and S^{35}-labelled T2 bacteriophage reveal that shortly after its attachment to the resin, the virus is split into its phosphorus-containing DNA, and the sulfur-containing protein fraction.

It is proposed that the splitting of phage into its protein and DNA compo-

nents that occurs ordinarily at the surface of a cell is a result of the establishment of the primary electrostatic bonds, just as in the attachment to cationic exchange resins.

The affinity of cationic resins for influenza virus is great enough to remove the virus almost completely from red cells to which it has become attached.

The technical assistance of Mr. Howard Lee is gratefully acknowledged.

BIBLIOGRAPHY

1. Puck, T. T., Garen, A., and Cline, J., *J. Exp. Med.*, 1951, **93,** 65.
2. Garen, A., and Puck, T. T., *J. Exp. Med.*, 1951, **94,** 177.
3. Tolmach, L. J., and Puck, T. T., *J. Am. Chem. Soc.*, 1952, **74,** 5551.
4. Garen, A., data to be published.
5. Abramson, H. A., Moyer, L. S., and Gorin, M. H., Electrophoresis of Proteins, New York, Reinhold Publishing Corp., 1942, 303.
6. Putnam, F. W., *Science*, 1950, **111,** 481.
7. Hershey, A. D., and Chase, M., *J. Gen. Physiol.*, 1952, **36,** 39.
8. Salk, J. E., *J. Immunol.*, 1944, **49,** 87.
9. Hirst, G. K., *J. Exp. Med.*, 1942, **76,** 83.
10. Muller, R. H., and Rose, H. M., *Proc. Soc. Exp. Biol. and Med.*, 1952, **80,** 27.
11. Ruegamer, W. T., data to be published.
12. Burnet, F. M., and Edney, M., *Australian J. Exp. Biol. and Med. Sc.*, 1952, **30,** 105.
13. Lo Grippo, G. A., *Proc. Soc. Exp. Biol. and Med.*, 1950, **74,** 208.